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**Hugo André do Monte
Dias Gomes**

**Extração de cafeína de borras de café utilizando
soluções aquosas de líquidos iónicos**

**Extraction of caffeine from spent coffee using
aqueous solutions of ionic liquids**



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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Engenharia Química, realizada sob a orientação científica do Professor Doutor João Manuel da Costa Araújo Pereira Coutinho, Professor Catedrático do Departamento de Química da Universidade de Aveiro e coorientação da Doutora Mara Guadalupe Freire Martins, Investigadora Coordenadora do Departamento de Química, CICECO, da Universidade de Aveiro.

À minha família e a todos que considero parte dela...

o júri

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Apesar destas palavras aparecerem praticamente no início desta dissertação sentir-me-ia incapaz de as escrever caso não as sentisse como as últimas e igualmente relevantes deste trabalho bem como de um percurso.

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Todas estas palavras poderiam facilmente ser substituídas por um singelo mas sentido Obrigado.

Palavras-chave

Extração sólido-líquido, biomassa, cafeína, líquidos iônicos, planeamento fatorial, hidrótopos.

Resumo

O principal objetivo desta dissertação recai sob o estudo de técnicas de extração alternativas de compostos de valor acrescentado a partir de biomassa, mais benignas e eficientes do que as habitualmente utilizadas. Em particular, estudou-se a utilização de soluções aquosas de líquidos iônicos (LIs) para a extração de cafeína a partir de borras de café. O interesse da extração deste composto deve-se às suas propriedades e consequente interesse pelas indústrias alimentar, farmacêutica, cosmética e agroquímica. De salientar ainda que as borras de café são um produto residual sem valor associado, constituindo uma matéria-prima praticamente sem custos e com um enorme potencial de compostos químicos de valor acrescentado passíveis de serem extraídos. Para tal, efetuaram-se extrações do tipo sólido-líquido a partir de borras de café utilizando várias soluções aquosas de LIs, assim como misturas de LIs com sais.

Começou-se pela síntese de líquidos iônicos próticos (LIPs) e sua caracterização. A utilização de LIPs prende-se com o facto de possuírem características muito singulares que facilitariam a recuperação bem como a separação dos compostos extraídos. Os resultados obtidos mostraram, que dos LIPs sintetizados, o mais promissor na extração de cafeína foi o acetato de trietanolamina. Mais concretamente, para uma concentração de 2 M, uma temperatura de 358 K (85°C), uma razão sólido-líquido de 0,1 e um tempo de extração de 45 minutos, conseguiu-se obter uma percentagem de cafeína extraída de 3,01% (m/m).

Também se estudou a otimização da extração da cafeína de borras de café utilizando como solvente soluções aquosas de diferentes LIs apróticos, com o catião colínio em comum, em que se recorreu a um planeamento fatorial por forma a obter o ponto ótimo de extração, relativamente à temperatura, razão sólido-líquido, tempo e concentração do LI. Verificou-se que o ponto ótimo de operação para a extração de cafeína com soluções aquosas de bicarbonato de colínio ocorre para um tempo de 30 minutos, uma temperatura de 323 K (50°C), uma razão sólido-líquido de 0,05 e concentração de LI de 1,5 M, onde a extração de cafeína obtida foi de 1,85% (m/m). Foram também testadas misturas de sais com atividade hidrotópica com LIs apróticos, com um catião imidazólio em comum, a fim de conjugar as propriedades de ambos os compostos de forma a potenciar a extração de cafeína. Constatou-se que os compostos (sal ou LI) que contêm o anião tosilato tem capacidade de extrair uma maior percentagem de cafeína. Os valores de extração obtidos, utilizando o LI tosilato de 1-butil-3-metilimidazólio ou uma mistura equimolar de tosilato de sódio com cloreto de 1-butil-3-metilimidazólio, foram semelhantes, sendo que para a concentração de 1M a extração de cafeína foi de 3,50 % (m/m), a uma temperatura de 348 K (75°C), tempo de 30 minutos e razão sólido-líquido de 1:10.

Em suma, esta tese descreve a aplicação de soluções aquosas LIs visando o desenvolvimento de processos extrativos mais eficientes e sustentáveis.

Keywords

Liquid-liquid extraction, biomass, caffeine, ionic liquids, factorial planning, hydrotropes.

Abstract

The main objective of this work consists on the development of a more benign and efficient technique for the extraction of added-value compounds from biomass. In particular, the use of ionic liquids (ILs) for the extraction of caffeine from spent coffee was investigated. This compound display important properties with relevance in food, pharmaceutical, cosmetic and agrochemical industries. Spent coffee grounds (SCG) are a waste product without commercial value, being thus a raw material with virtually no cost and with a huge potential of value-added compounds able to be extracted. To this end, solid-liquid extractions from biomass were carried out using aqueous solutions of various ILs as well as mixtures of ILs and salts.

As a first attempt, several protic IIs (PIIs) were synthesized and characterized. These were chosen since they have unique characteristics that facilitate their recovery as well as the separation of the extracted components. The results obtained indicate that triethanolammonium acetate is the best candidate to extract caffeine. More specifically, at the concentration of 2 M, a temperature of 358 K (85°C), a solid-liquid ratio of 0,1 and 45 minutes of extraction time, it was achieved a value of extracted caffeine of 3.01% (w/w).

Aqueous solutions of different aprotic ILs, with a common cholinium cation, were also investigated for the extraction of caffeine. A factorial planning was carried out in order to identify the optimum operating conditions. The optimum operating conditions for caffeine extraction with aqueous solutions of cholinium bicarbonate were obtained in the following conditions: time of 30 minutes, temperature of 323 K (50°C), solid-liquid ratio of 0.05 and LI concentration of 1.5 M, where the value of caffeine extraction obtained was of 1.85% (w/w).

Mixtures of salts with hydrotropic characteristics with ILs were finally investigated for the extraction of caffeine in order to combine the properties of both classes of compounds. It has been found that the compounds containing the tosylate anion have a higher ability to extract caffeine. In addition, the LI 1-butyl-3-methylimidazolium tosylate or an equimolar mixture of sodium tosylate with 1-butyl-3-methylimidazolium chloride, lead to similar values of extracted caffeine. More specifically, for a concentration of 1M, the value obtained for the extraction of caffeine was of 3.50% (w/w), at a temperature of 348 K (75°C), extraction time of 30 minutes and solid-liquid ratio of 1:10.

In summary, this thesis describes the application of aqueous solutions of ILs foreseeing the development of more efficient and sustainable extractive processes.

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List of symbols

wt % – weight percentage (%);

λ – wavelength (nm);

Abs – absorbance (dimensionless);

M_w – molecular weight ($\text{g}\cdot\text{mol}^{-1}$);

R^2 – correlation coefficient (dimensionless).

List of abbreviations

IL – ionic liquid;
UV – ultraviolet;
VOC – volatile organic compound;
[Ch][Ac] – (2-hydroxyethyl)trimethylammonium (cholinium) acetate;
[Ch][But] – (2-hydroxyethyl)trimethylammonium (cholinium) butanoate;
[Ch]Cl – (2-hydroxyethyl)trimethylammonium (cholinium) chloride;
[Ch][Pro] – (2-hydroxyethyl)trimethylammonium (cholinium) propanoate;
[C₄mim]Cl – 1-butyl-3-methylimidazolium chloride;
[C₄mim][N(CN)₂] – 1-butyl-3-methylimidazolium dicyanamide;
[C₄mim][Tos] – 1-butyl-3-methylimidazolium tosylate;
[C₄mim][CF₃SO₃] – 1-butyl-3-methylimidazolium trifluoromethanesulfonate;
[C₄mpy]Cl – 1-butyl-3-methylpyridinium chloride;
[EA][CH₃CO₂] – ethanolammonium acetate;
[DEA][CH₃CO₂] – diethanolammonium acetate;
[TEA][CH₃CO₂] – triethanolammonium acetate;
[Bic]⁻ – bicarbonate (HCO₃⁻);
[Pro]⁻ – propanoate (C₂H₅COO⁻);
[But]⁻ – butanoate (C₄H₇O₂⁻);
[DHCit]⁻ – dihydrogen citrate;
[DHPh]⁻ – dihydrogen phosphate;
SCG – spent coffee grounds;
PIL – protic ionic liquid;
ppm – part per million.

1. General introduction

1.1. Scope and objectives

Coffee is the most important agricultural commodity in the world and is in a second place after petroleum in what concerns global trade activity and value [1]. In Portugal, where coffee consumption is so deeply rooted in cultural habits, the total imports of coffee in 2006 were over 59 000 tons [2]. However, the production of drinkable coffee leads to large amounts of waste, namely spent coffee. This residue has however no commercial value although it is rich in several added-value compounds and caffeine.

The main purpose of this thesis consists on the investigation of ionic liquids (ILs) as a new class of extractive solvents for caffeine from spent coffee grounds (SCG). Caffeine displays a wide variety of relevant properties, namely, antioxidant, anti-inflammatory, radical scavenger and antimicrobial properties [3], currently having special relevance in food, dietary, pharmaceutical and agrochemical industries.

Caffeine-containing drinks are very popular around the world, and recently, the Food and Drug Administration (FDA) regulated that any added caffeine must be labelled on human consumption products owing to possible adulteration and health concerns [4]. Another worthy example is the current controversy that exists regarding the consumption/use of natural *versus* synthetic compounds [5]. Most consumers prefer natural compounds since the safety of synthetic counterparts is not fully known [5]. Based on these human apprehensions, the extraction of value-added compounds or fine chemicals from natural sources has gained a tremendous importance in the past few years [6]. However, conventional extraction processes used to this end still present several drawbacks, such as a low efficiency and non-selectivity, are time-consuming, may require a high energetic input, or lead to the degradation of the targeted compounds. Furthermore, traditional methods generally involve the use of large amounts of volatile organic solvents leading to safety and environmental concerns. Moreover, the use of hazardous solvents may prevent the high-value chemicals to be used for human consumption [7].

The traditional approaches developed for the extraction of caffeine from biomass involve solid-liquid extractions using organic solvents and supercritical carbon dioxide [8]. Foreseeing more efficient and low cost techniques for the extraction of caffeine from biomass, solid-liquid extractions using aqueous solutions of ILs, as well as mixtures of IL with salts were investigated.

In this work, the first approach consisted on the extraction of caffeine from SCG using aqueous solutions of protic ILs (PILs) and one aprotic IL, for comparison purposes. Three PILs were synthesized with the goal of replacing the more expensive and less benign imizadolium-based ones. The operational conditions, such as the influence of the IL structural features, temperature,

concentration of IL, time of extraction and solid-liquid ratio were evaluated, using a experimental factorial design for this purpose. Nevertheless, there are some concerns associated with the use of ILs in human health, and thus the application foreseen for this extracted caffeine will be as a biopesticide. An alternative scenario for the most studied and moderately toxic ILs consists on the use of non/less toxic ILs, such as the cholinium-based. Thus, as a second approach, aqueous solutions of cholinium-based ILs were also investigated for the extraction of caffeine from SCG. An experimental factorial design was also performed to evaluate the best operational conditions. The last stage of this work addresses the study of aqueous mixtures of ILs and salts which act as hydrotropes. The effects of the hydrotrope chemical structure and concentration of ILs and salts were evaluated. The extraction mechanism as a result of an improved solubility phenomenon, which is mostly governed by the formation of IL/salts-solute aggregates, is also presented and discussed.

1.2. Spent coffee grounds (SCG)

Spent coffee grounds (SCG) are obtained from the treatment of coffee powder with hot water to prepare coffee and constitute the main residue of the coffee industry that yearly generates 6 million tons of this residue [9]. Most SCG are not valorised, being discharged or burned [10]. However, SCG are rich in organic matter, as well as in other compounds, such as oils, caffeine, tannins and polyphenols [10]. Thus, these residues can be explored as a rich source of valuable compounds before their discharge. In a recent study, extracts from SCG exhibited anti-tumor and anti-allergic activities, which were related to the presence of phenolic compounds in their composition [11], such as chlorogenic acid (this being one of the most abundant phenolic compounds (PhC) in SCG) [11, 12]. SCG have still a significant amount of caffeine (values of 4.526 mg·g⁻¹ have been reported [13]).

1.2.1. Caffeine

Caffeine (Figure 1.1) is a chemical compound which is naturally found in coffee, and in other plant sources, such as cocoa, tea, cola nuts, guarana seeds, etc. [14]. Chemically, it can be classified as belonging to the heterocyclic group of compounds called purines. Caffeine can also be classified as an alkaloid. In its pure state it appears as a white powder with a bitter taste but odorless [15].

The physical properties of caffeine are listed in Table 1.1.

Table 1.1. Physical properties of caffeine

Molar mass (g·mol ⁻¹)	Density at 298.15 K (g·cm ⁻³)	Melting point (K)	Water solubility at 298.15 K (g·dm ⁻³)	pKa at 298.15 K
194.2	1.23	511.15	21.7	0.6

Caffeine is soluble in organic solvents, such as alcohols and chloroform, and mildly soluble (20 g·dm⁻³) in water at room temperature [16]. It was first isolated and purified in 1819 by the German Chemist Friedrich Ferdinand Runge [17].

Caffeine exhibits pharmacological properties, more specifically it is an adjuvant capable of enhancing the analgesic effect of aspirin and other drugs [18], stimulates the release of epinephrine (adrenaline) and dopamine, which act to increase heart rate, blood pressure, urine production, and the release of glucose into the blood from the liver [19]. Furthermore, it is currently used in cosmetic applications due to recent described properties on the combat of skin cancer [19] and hair strengthening [20], as well as in nutrition applications in many existing food supplements [21]. Therefore, the caffeine extraction from natural sources, such as spent coffee grounds, through economic and sustainable processes is of great practical interest for further applications of this natural alkaloid [22].

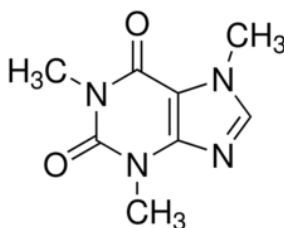


Figure 1.1. Molecular structure of caffeine.

1.3. Caffeine extraction methods

Nowadays, there is a growing interest in the extraction and purification of natural compounds for applications in the food, cosmetic and pharmaceutical industries. In addition, and due to sustainability concerns, it is desired to use sub-products or even wastes aiming their valorization. Currently, caffeine extraction (essentially for decaffeination purposes and human intake) is carried out using supercritical carbon dioxide or related mixtures using co-solvents, such as alcohols and water, to improve the extraction yields [23]. Another possible approach consists on the use of organic solvents [24]. The last option consists on solid-liquid extractions, being this technique the oldest unit operations in the chemical industry. In these, the choice of the solvent is carried out taking into consideration its selectivity, capability for dissolving the solute, density, viscosity, surface tension, toxicity, boiling temperature, chemical and thermal stabilities and cost [24]. Obviously, due to the toxicity of some organic solvents, there are some restrictions to their use in the food, cosmetic and pharmaceutical industries [25]. The most investigated organic solvents are chloroform, methylene chloride and ethyl acetate. Water as also been studied as well as water-organic solvent mixtures (water-ethanol, water-methanol and water-acetone) [26, 27]. These methods have however some disadvantages since they require several hours of extraction and result in low yields of caffeine [25]. Furthermore, the organic compounds commonly employed display major drawbacks in what concerns safety and environmental issues.

In recent years, ILs emerged as alternative solvents for separation processes [28]. In particular, the application of ILs for the extraction of essential oils from orange peels, in the reactive dissolution of star anise seeds towards the isolation of Shikimic acid for the production of Tamiflu, in the sequestration of suberin from cork, in the extraction of lactones, tannins and phenolic compounds from medicinal plants, in the extraction of the bioactive alkaloid glaucine from plant material, in the isolation of pharmaceutically active betulin from birch bark and in the pre-treatment of lignocellulosic biomass [24] have been reported. In particular, aqueous solutions of

imidazolium-based ILs have also been investigated for the extraction of caffeine from guaraná seeds [29].

1.4. Ionic Liquids

In 1914, when Paul Walden was testing new explosives for the substitution of nitroglycerin, it was synthesized the first IL, ethylammonium nitrate ($[\text{CH}_3\text{CH}_2\text{NH}_3][\text{NO}_3]$), with a melting point of 285.15 K (12°C) [30]. However, only in 1934, the first patent regarding an industrial application of ILs was filled for the preparation of cellulose solutions, by Charles Graenacher [31]. During the 2nd World War, new patents involving the use of ILs in mixtures of aluminium chloride (III) and 1-ethylpyridinium bromide for the electrodeposition of aluminium were filled [32]. Nevertheless, only in the past few years, with the appearance of air and water stable ILs, the research and development of novel ILs and their possible applications increased significantly.

ILs are ionic compounds (with a melting temperature below 373 K) that belong to the molten salts group; often, they are called room temperature ILs when they are liquid at temperatures close to room temperature [33]. They are usually constituted by a large and organic cation and an organic or inorganic anion [33]. The low melting temperatures of ILs are typically associated with the lack of an ordered crystalline structure [34], a result of the weak intermolecular interactions derived from the large size ions and their charge distribution [33, 34]. The ionic nature of ILs is responsible for some of their unique properties, namely a negligible vapour pressure under atmospheric conditions, low flammability, high thermal and chemical stabilities, a large liquid temperature range, improved selectivity, high ionic conductivity, excellent microwave-absorbing ability, and a high solvating aptitude for organic and inorganic compounds [35, 36]. All these properties made them improved alternatives to more typical solvents used nowadays in academia or industry, more specifically to VOCs. Currently, ILs are used in a wide range of applications, as in organic chemistry (Suzuki reaction [37]) and chemical reactions [38], as well as in new materials chemistry (electrolytes for the electrochemical industry [39]). ILs are also applied in inorganic synthesis, polymerization [40], and in batteries and fuel cells investigations [41]. Beyond these applications, ILs have also been used in biphasic chemical processes [42-45] or in biomass processing [46, 47].

Amongst the large range of ILs that can be synthesized, the most commonly studied are nitrogen-based ILs with their general cation structures presented in Figure 1.2. The cation can be highly complex with alkyl side chains of different length, diverse substitution positions and also additional functional groups [48]. Furthermore, the anion can be of a very different chemical nature, such as halogens, sulphates, cyano-based, fluorinated, etc. Thus, there is an extremely wide

range of possible anion and cation combinations taken from a pool of organic and inorganic ions. Consequently, ILs are often considered as tunable “designer solvents”, meaning that their properties can be tuned by varying the structure of the component ions to obtain desired characteristics [49, 50].

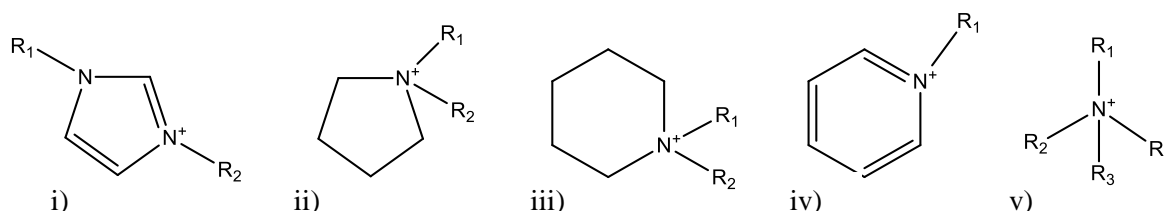


Figure 1. 2. Cation structures of nitrogen-based ILs: (i) dialkylimidazolium, (ii) dialkylpyrrolidinium, (iii) dialkylpiperidinium, (iv) alkylpyridinium, and (v) tetralkylammonium.

Besides the existence of over six hundreds of common solvents used in industry, most aprotic ILs are an innovative ambient-friendly alternative due to their unique combination of non-volatility and non-flammability [51-54]. In the environmental field, and with the intent of diminishing the air pollution, ILs are improved alternatives to VOCs due to their negligible vapour pressure. This is the main reason behind the categorization of ILs as “green solvents” [55]. However, the fact of displaying a negligible vapour pressure is not enough to assure that these compounds are in fact “green” [55, 56]. Properties such as toxicity and biodegradability must be accessed before such assumptions. For instance, even the most hydrophobic ILs have a non-negligible miscibility with water, which can result in the contamination of aqueous streams [57, 58]. In recent years, several studies were conducted to evaluate the toxicity and biodegradability of ILs [59-61], either by the combination of different anions and cations or by changing the alkyl side chain length and number of alkyl groups at the cation ring. One of these studies showed that the ILs toxicity is primordially determined by the cation nature and increases with the increase of the length of the alkyl side chain (increase in hydrophobicity) [58, 62]. Commonly, the anion has a smaller influence on toxicity than the cation, and generally, short cation alkyl chains or more hydrophilic ILs display lower toxicity [63]. The solubility of ILs in water decreases with their hydrophobicity, *i.e.*, the more toxic ILs are those that usually exhibit lower mutual solubilities with water, and hence, their environmental impact in aquatic streams is somewhat minimized [58].

2. Extraction of caffeine using protic ILs aqueous solutions

2.1. Introduction

Based on the tailoring ability of ILs, either for the solvation of a wide array of compounds or to extract the most diverse biomolecules from biomass [64], in this work, aqueous solutions of protic ILs (PILs) were studied to extract caffeine from SCG. Recent investigations reported the use of PILs in the extraction of tannins [65] and benzofuranoids [66] from biomass. However, most studies report to the use of aprotic ILs for the extraction of biomolecules from biomass [67], while one work aiming the extraction of caffeine using imidazolium-based ILs [29].

Most of the investigated aprotic ILs for the extraction of added-value compounds from biomass are imidazolium-based [67], and these tend to be more expensive and less benign, biocompatible and biodegradable when compared to protic ILs. In addition, protic ILs can usually be recovered and reused by distillation [68]. Although the non-volatile nature (and the consequent no contamination of the atmosphere) of the protic ILs is lost, it should be remarked that their low vapour pressure at atmospheric conditions, allied to a proper manipulation and design of the extraction process, as well as the easy IL recovery [69], can bring significant improvements on the reduction of the process footprint on the environment [67]. Thus, the major goal of this work consists on the replacement of imidazolium-based ILs commonly used for extraction approaches by PILs.

The synthesis of PILs occurs *via* proton exchange in stoichiometric conditions through the reaction of a primary amine and a Brønsted acid [70]. These reagents (acid and base) have a low cost and the synthesis of PILs is very simple, and thus the production of PILs for further applications is much simpler and economical when compared with their aprotic IL analogues.

SCG is a residue with almost no commercial value with still a significant amount of caffeine [71]. In order to valorize this residue, aqueous solutions of three PILs and one imidazolium-based IL were here used as extractive solvents for caffeine. The operation conditions were further optimized by a factorial planning.

2.2. Experimental section

2.2.1. Chemicals

The spent coffee grounds were supplied by the *Cafeteria* of the Chemistry Department of University of Aveiro, in Portugal. The samples were dried until constant weight (\approx for 6 days at 373 K). SCG have a humidity content of (59.52 ± 2.53) %. After this step, SCG with particles diameter (d) < 0.4 mm were isolated.

The solvents used for the solid-liquid extraction of caffeine from SCG were water and aqueous solutions of four ILs. The IL, $[\text{C}_4\text{mim}][\text{CH}_3\text{CO}_2]$, (95 wt % pure), was purchased from Iolitec, whereas the three PILs, namely ethanolammonium acetate, $[\text{EA}][\text{CH}_3\text{CO}_2]$, diethanolammonium acetate, $[\text{DEA}][\text{CH}_3\text{CO}_2]$ and triethanolammonium acetate, $[\text{TEA}][\text{CH}_3\text{CO}_2]$, were synthesized in your lab according to standard protocols [72]. Their purities were confirmed by ^1H and ^{13}C NMR spectra. All ILs samples were dried for at least 48 h, under vacuum and at a room temperature (≈ 298 K), before use. The water content of all ILs, after the drying procedure, was < 1000 ppm as determined by Karl-Fischer titration. The chemical structures of the studied ILs are depicted in Figure 2.1.

For the synthesis of PILs, several amines and acetic acid were used to obtain ethanolammonium-based cations with the common acetate anion. Ethanolamine (99%), diethanolamine (99%), triethanolamine (99%) and acetic acid (99.99%) were purchased from Sigma-Aldrich and used as received.

The extraction of caffeine using soxhlet extractions was also carried out using ethanol, dichloromethane and petroleum ether. Petroleum ether, dichloromethane and ethanol were purchased from Sigma-Aldrich, BDH Prolabo and Carlo Erba, respectively, with 99.9 % of purity.

Double distilled water, passed through a reverse osmosis system, and further treated with a Milli-Q plus 185 water purification equipment, was used in all experiments.

The high purity caffeine, used as a standard in the calibration curves, was obtained from Marsing & Co. Ltd., with a nominal purity ≥ 98.5 wt%.

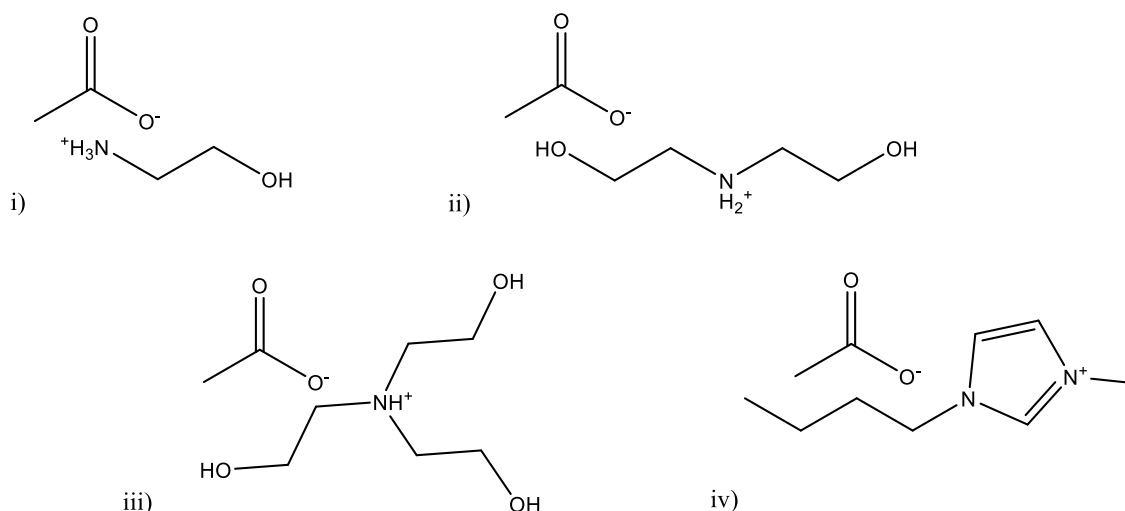


Figure 2.1. Chemical structures of the ILs used in the extraction of caffeine from SCG: (i) [EA][CH₃CO₂]; (ii) [DEA][CH₃CO₂]; (iii) [TEA][CH₃CO₂] and (iv) [C₄mim][CH₃CO₂].

2.2.2. Experimental procedure

2.2.2.1. Synthesis of protic ILs

Two hundreds mmol of amine were placed in a 100 mL glass vial and cooled using an ice bath under vigorously stirring. Into this cold solution, two hundreds mmol of acetic acid were added drop-wise while maintaining the temperature at 273.15 K since this reaction is exothermic. The mixture was then allowed to reach room temperature and kept under constant stirring for 12h. To reduce the volatile impurities to negligible values, all synthesized PILs were dried under constant agitation at vacuum and room temperature (298 K) for 48 h.

2.2.2.2. Characterization of PILs

The synthesized PILs were characterized using several techniques, namely ¹H and ¹³C NMR, density, viscosity, refractive index and melting temperature. The water content of each IL, after the drying step and immediately before the measurements of the thermophysical properties was determined by Karl Fischer titration using a Metrohm 831 Karl Fischer coulometer and was found to be < 1000 ppm.

The density and viscosity of the pure PILs were measured using an automated SVM 3000 Anton Paar rotational Stabinger viscometer-densimeter in the temperature range from (318.15 to 368.15) K, with a temperature uncertainty of ± 0.02 K. The dynamic viscosity has a relative uncertainty within 0.35 %, while the absolute uncertainty in density is $\pm 5 \times 10^{-4} \text{ g}\cdot\text{cm}^{-3}$.

Measurements of refractive index (n_D) were performed at 589.3 nm using an automated Abbemat 500 Anton Paar refractometer, developed for measuring both liquid and solid samples. The Abbemat Anton Paar refractometer uses reflected light to measure the refractive index, where the sample on the top of the measuring prism is irradiated from different angles by a LED. Refractive index measurements were carried out in the temperature range (288.15 to 353.15) K at atmospheric pressure. The maximum deviation in temperature is ± 0.01 K and the maximum uncertainty in the refractive index is ± 0.00002 .

The melting temperatures of PILs were determined using a BX51 Olympus polarized optical microscope (Olympus Co., Tokyo, Japan) equipped with a LTS120 Linkam temperature-controlled stage (Linkam Scientific Instruments, Ltd., Tadworth, U.K.) operating between 248.15 and 353.15 K. The PILs samples (2mg, approximately) were placed under argon, in concave slides with coverslips sealed with thermal joint silicone, cooled to 248.15 K, and allowed to stay at this temperature for 30 min. After this step, the samples were subjected to a 0.1 K min^{-1} heating run. The uncertainty in the melting temperature obtained from POM measurements was taken as not higher than 1.0 K.

2.2.2.3. Caffeine Extraction

All aqueous solutions were prepared gravimetrically within 10^{-4} g (using an analytical balance Mettler Toledo Excellence - XS205 Dual Range). Mixtures of specific amounts of SCG and aqueous solutions were also prepared by weight and in sealed glass vials. Several concentrations of PILs and different solid-liquid ratios, times of extraction and temperatures were investigated to improve the extraction of caffeine according to the surface response methodology described below.

The extractions were carried out in commercial Carousel Radleys Tech equipment able to both stirring and maintain the temperature within ± 0.5 K. In all experiments the stirring was kept constant at 300 rpm. After extraction, the overall solution was filtered under vacuum using a $0.45 \mu\text{m}$ cellulose membrane. After filtration, the extracted liquid solution was quantified through UV-spectroscopy, using a SHIMADZU UV-1700, Pharma-Spec Spectrometer, at a wavelength of 274 nm using a calibration curve previously established for caffeine. The effect of each IL on the quantification technique was evaluated and it was found to be of no major influence taking into account the dilutions carried out.

The amount of caffeine present in the SCG was calculated according to the weight of pure caffeine present in the extract divided by the total weight of dried biomass.

Soxhlet extractions with organic solvents were also conducted during 7 hours (420 min). For the UV-spectroscopy quantification additional calibration curves were established in the different solvents, while caffeine was quantified at $\lambda = 274$ nm.

2.2.2.4. Surface Response Methodology

Factorial planning allows the simultaneous analysis of various factors and more than one answer [73]. In the 2^k factorial planning there are k factors that can contribute to a different response. This planning is defined by the central point (zero level), the factorial points (1 and -1, level one) and the axial points (level α). The center point and the axial points are defined by the user depending on the purpose of the work. The axial points are encoded at a distance α from the central point [73]:

$$\alpha = (2^k)^{\frac{1}{4}} \quad (1)$$

Data are treated according to a second order polynomial equation:

$$y = \beta_0 + \sum_i \beta_i X_i + \sum_i \beta_{ii} X_i^2 + \sum_{i < j} \beta_{ij} X_i X_j \quad (2)$$

where y is the response variable and β_0 , β_i , β_{ii} and β_{ij} are the adjusted coefficients for the intercept, linear, quadratic and interaction terms, respectively, and X_i and X_j are independent variables. This mathematical model allows the drawing of surface response curves and through their analysis the optimal conditions can be determined.

In this work, it was used a 2^3 factorial planning with the goal of optimizing the amount of extracted caffeine from SCG as well as to identify the most significant parameters and their interactions. The 2^3 factorial planning is provided in appendix A in Table A.1. The obtained results were statistically analysed with a confidence level of 95%. The Student's t-test was used to check the statistical significance of the adjusted data [73]. The adequacy of the model was determined by evaluating the lack of fit, the regression coefficient (R^2) and the F-value obtained from the analysis of variance (ANOVA) that was generated. Three-dimensional surface response plots were generated by varying two variables within the experimental range and holding the other factors constant at the central point.

The Statsoft Statistica 8.0[®] software was used for all statistical analysis, and Matlab R2010a, The MathWorks, was used for representing the response surfaces and contour plots.

2.3. Results and discussion

2.3.1. Density, Viscosity, Melting Temperatures and Refractive Index Measurements

In this work, three PILs were synthesized aiming at replacing the commonly used imidazolium-based ILs for the extraction of value-added compounds from biomass. They were characterized according to their NMR spectra (Appendix B.1, Figure B. 1.), density, viscosity, refraction index and melting temperatures. Density, viscosity, and refractive index of the synthesized PILs are given in Figures 2.2 to 2.4. The detailed data are given in Appendix B.1 (Table B.1).

For all ILs, the density decreases with an increase in temperature. In addition, the density of the [DEA]-based ILs is similar to that displayed by the [TEA]-based compound at the same conditions. However, there is a significant decrease of density when the ammonium cation presents only one hydroxyl group, namely with the [EA]-based IL. In the literature [74], some properties for acetate-based ILs have already been reported. Comparing the experimental data obtained in this work (Figure 2.2) and those reported in the literature [74] for a given temperature, for instance at 298.15 K, the density decreases in the following order: [TEA][CH₃CO₂] \approx [DEA][CH₃CO₂] > [EA][CH₃CO₂] > [C₂mim][CH₃CO₂] > [C₄mim][CH₃CO₂] > [C₂mim][CH₃CO₂] > [C₄mpyr][CH₃CO₂] > [N₀₁₁₂][CH₃CO₂], meaning that protic ILs are more dense than more conventional ILs based on imidazolium and pyrrolidinium cations combined with the acetate anion. In addition, the increase in the number of hydroxyl groups attached to the ammonium cation increases the density of the IL.

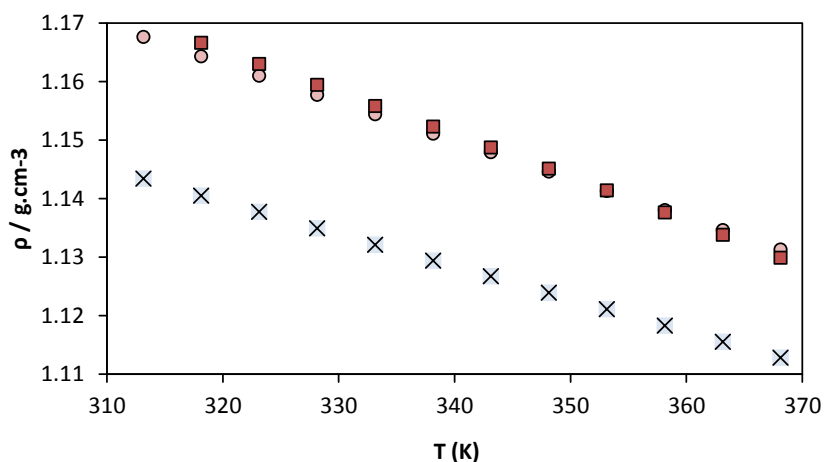


Figure 2.2. Experimental density (ρ) of: (×) [EA][CH₃CO₂], (○) [DEA][CH₃CO₂]; (■) [TEA][CH₃CO₂].

The refractive index specifies the dielectric response to an electrical field induced by electromagnetic waves (light) and is thus an optical property of materials. The experimental refractive index data of the measured ILs are depicted in Figure 2.3. The refractive index was

measured upward and downward on temperature, with no hysteresis effects observed, and within the temperature range from 283.15 to 353.15 K. For all ILs, the refractive index decreases with the temperature increase. The refractive index values for the $[\text{CH}_3\text{CO}_2]$ -based ILs, making use of already published data [74] decrease in the following sequence: $[\text{C}_2\text{mim}][\text{CH}_3\text{CO}_2] > [\text{C}_4\text{mim}][\text{CH}_3\text{CO}_2] > [\text{TEA}][\text{CH}_3\text{CO}_2] > [\text{DEA}][\text{CH}_3\text{CO}_2] > [\text{EA}][\text{CH}_3\text{CO}_2] > [\text{C}_4\text{mpyr}][\text{CH}_3\text{CO}_2] > [\text{N}_{0112}][\text{CH}_3\text{CO}_2] > [\text{C}_2\text{im}][\text{CH}_3\text{CO}_2]$.

Densities and refractive indices for $[\text{EA}][\text{CH}_3\text{CO}_2]$ and $[\text{DEA}][\text{CH}_3\text{CO}_2]$ were also found in literature [72] and are in close agreement with our data with a relative deviation between 0.2% and 2.7%.

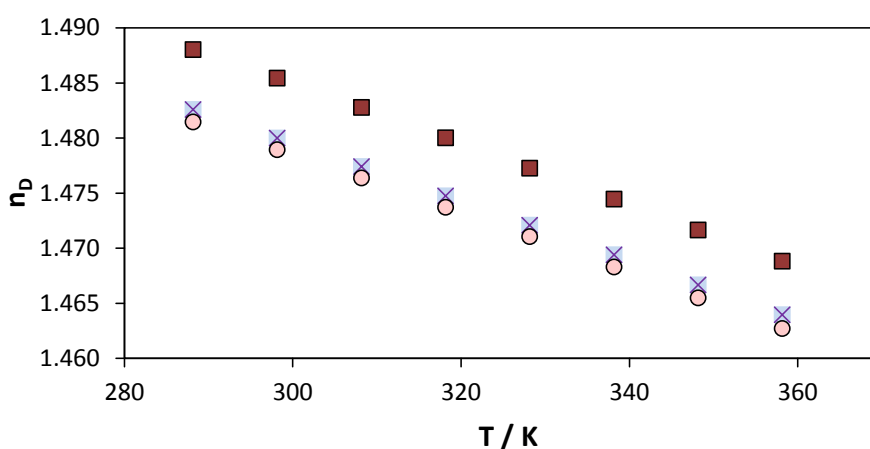


Figure 2. 3. Experimental refraction index of: (×) $[\text{EA}][\text{CH}_3\text{CO}_2]$, (○) $[\text{DEA}][\text{CH}_3\text{CO}_2]$; (■) $[\text{TEA}][\text{CH}_3\text{CO}_2]$.

Viscosity is the internal resistance of a fluid to a shear stress, and, in general, ILs present higher viscosities than conventional molecular solvents. Viscosity is an important property of ILs as it influences ionic conductivity and mass transport phenomena. The viscosity of the IL also influences the mass transfer during extractions.

The experimental viscosity data for the investigated ILs are depicted in Figure 2.4. At all studied temperatures, the viscosity of $[\text{EA}][\text{CH}_3\text{CO}_2]$ is similar to that displayed by $[\text{DEA}][\text{CH}_3\text{CO}_2]$. However, contrarily to that observed with density, the addition of one more hydroxyl group (from $[\text{DEA}]^+$ to $[\text{TEA}]^+$) leads to a decrease in the viscosity. Comparing the experimental data obtained in this work and those reported in the literature [74] for a given temperature, for instance at 298.15 K, viscosity decreases in the following sequence: $[\text{EA}][\text{CH}_3\text{CO}_2] \approx [\text{DEA}][\text{CH}_3\text{CO}_2] > [\text{TEA}][\text{CH}_3\text{CO}_2] > [\text{C}_4\text{mim}][\text{CH}_3\text{CO}_2] > [\text{C}_2\text{mim}][\text{CH}_3\text{CO}_2] > [\text{C}_4\text{mpyr}][\text{CH}_3\text{CO}_2] > [\text{N}_{0112}][\text{CH}_3\text{CO}_2] > [\text{C}_2\text{im}][\text{CH}_3\text{CO}_2]$, meaning that protic ethanolamine-based ILs are more viscous than imidazolium- and pyrrolidinium-based.

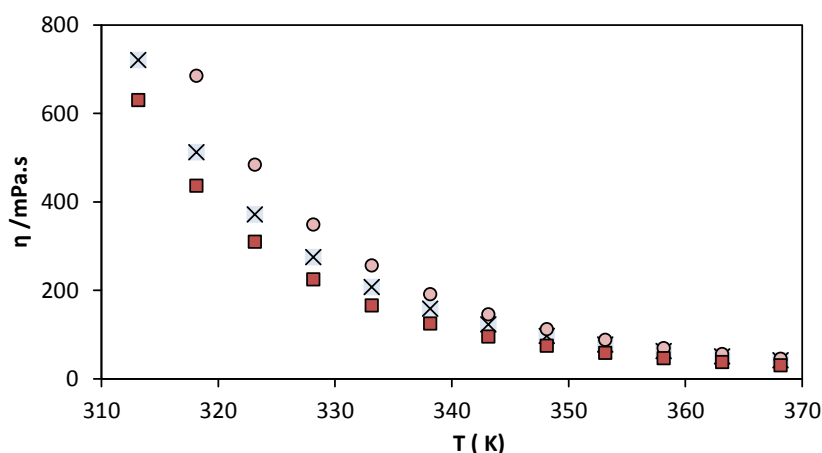


Figure 2. 4. Experimental viscosity Of: (x) [EA][CH₃CO₂], (○) [DEA][CH₃CO₂]; (■) [TEA][CH₃CO₂].

Finally, the melting point of each PIL was determined using a Light-Polarized Optical Microscopy (POM), and as previously validated by us [75]. Since [DEA][CH₃CO₂] always remained liquid at the temperature range investigated it was only possible to determine the melting temperatures of the two other PILs. The melting point of [EA][CH₃CO₂] is 334 ± 1 K ($61 \pm 1^\circ\text{C}$) and of [TEA][CH₃CO₂] it is 349 ± 1 K ($76 \pm 1^\circ\text{C}$).

2.3.2. Optimization of the operational conditions using water as the main solvent: response surface methodology

With the goal of optimizing the operational conditions to obtain high extraction yields, we used a response surface methodology (RSM). This type of strategy allows the exploitation of the relationship between the response (yield of caffeine) and the independent variables that can influence the extraction. In this work, a 2^3 factorial planning (presented in appendix A, Table A.1) was carried out with pure water and the temperature, the solid-liquid ratio and time of extraction were optimized. The central point used in the planning was 343 K (70°C), a solid-liquid ratio of 1:10 and a time of 30 min (the conditions used for the extraction of caffeine are presented in appendix A, Table A.2).

According to the statistical analysis (Figure 2.5) and the data depicted in Figure 2.6 it is evident that temperature is the most significant parameter leading to a region of maximum yield of extraction. Higher extraction temperatures are profitable for the extraction of caffeine. Moreover, an increase in temperature contributes to a decrease in viscosity of the IL solutions and to enhance their swelling ability. The solid-liquid ratio also leads to different yields of caffeine. In general, the

amount of extracted caffeine increases with the solvent volume. The time was the variable with the weakest influence in the extraction of the alkaloid. From the optimization approach, and using water as the main solvent, it can be concluded that the optimum conditions required for an effective extraction of caffeine are: temperature at 358 K (85°C), 45 min of extraction and a of solid-liquid ratio 1:10. Under these optimum operational conditions, the maximum extraction yield of caffeine using pure water was of 1.50 % (w/w).

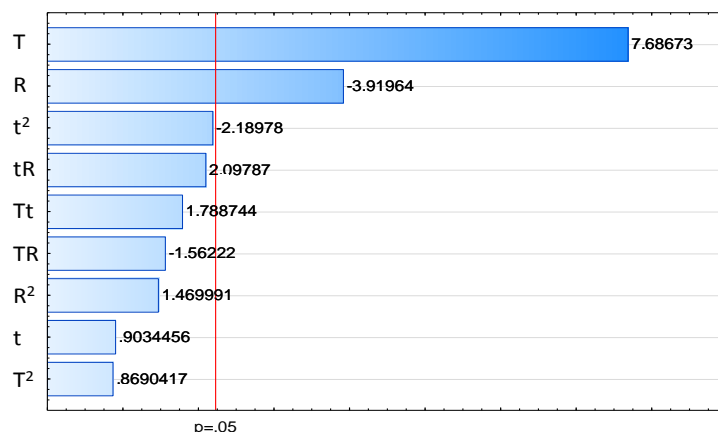


Figure 2. 5. Representation of optimization variables: (I) T (temperature); (II) R (ratio solid-liquid); and (III) t (time).

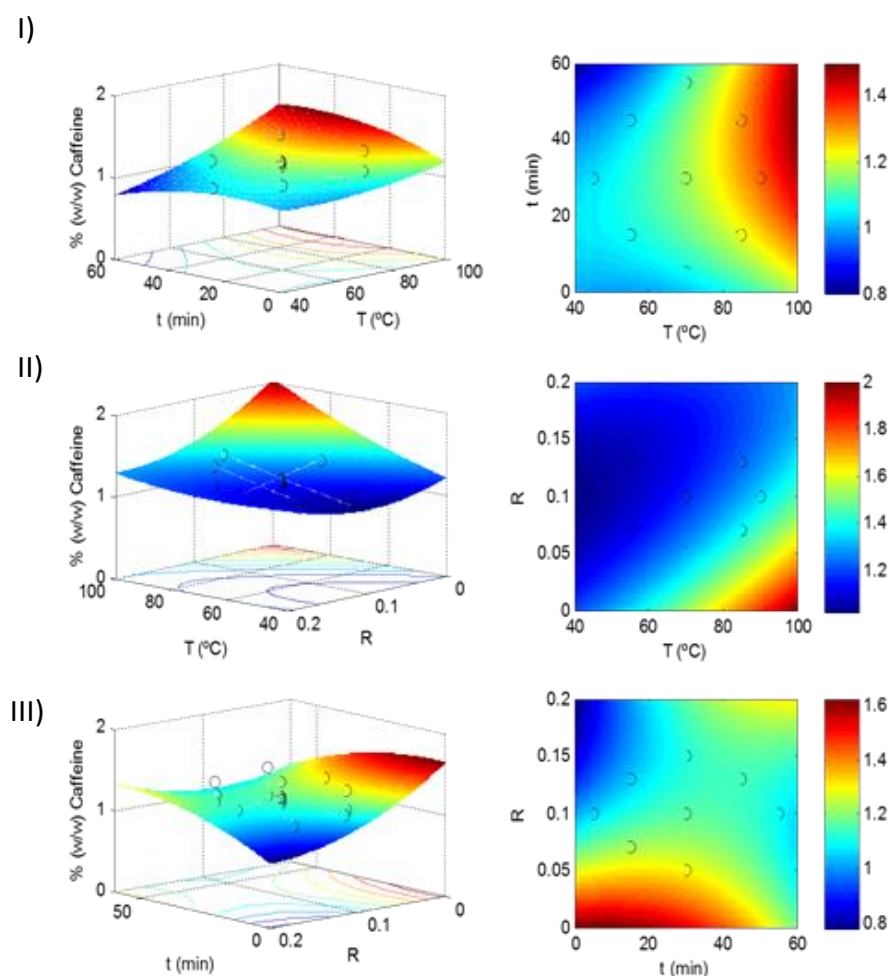


Figure 2. 6. Response surface plots (left) and contour plots (right) on the yield of caffeine using water as solvent: (I) T and t; (II) R and T; and (III) t and R.

2.3.3. Effect of the Ionic Liquid Structure

The ILs synthesized and investigated in this work consist on alkylethanolammonium cations combined with the acetate anion. One imidazolium IL with the acetate anion was also studied for comparison purposes, namely $[C_4mim][CH_3CO_2]$.

The results reported in Figure 2.6 show that high temperatures, a low solid-liquid ratio, and extraction times higher than 45 min are the optimal conditions for the extraction with water. Therefore, these conditions were used to evaluate the extraction performance of caffeine from SCG using aqueous solutions of ILs (at a 2M concentration). Pure water was also used at the same operational conditions for comparison purposes. The fixed operational conditions were solid-liquid ratio of 1:10, 358 K (85°C) and 45 min (with particles diameter < 0.4 mm).

Figure 2.7 reveals that all IL solutions perform better than pure water. In addition, the extraction of caffeine increases according to the following rank: water < [EA][CH₃CO₂] < [DEA][CH₃CO₂] < [C₄mim][CH₃CO₂] < [TEA][CH₃CO₂]. For PILs, in general, the extraction yield increases with the number of hydroxyl groups at the cation of the PIL. Remarkably, an extraction yield of caffeine of 3.01 w/w% was achieved with the aqueous solutions of [TEA][CH₃CO₂], confirming that protic ILs can be better extractive solvents than their more expensive and less-benign imidazolium-based counterparts.

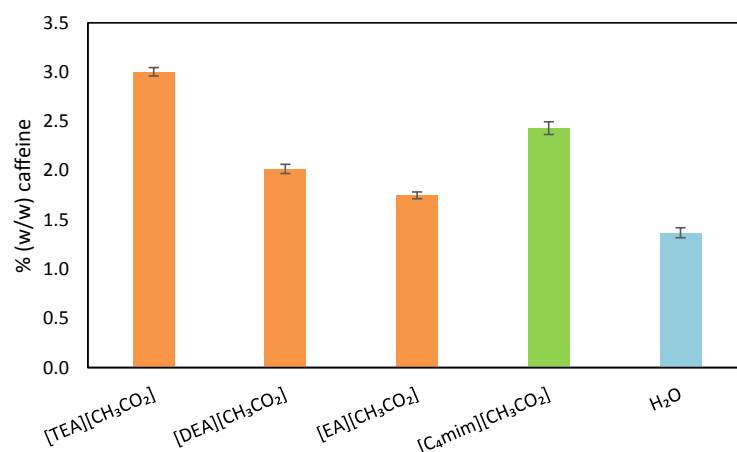


Figure 2. 7. Yield of caffeine extracted from SCG with different ILs ([IL] = 2 M, T = 85°C (358 K), S/L ratio = 1:10 and t = 45 min).

The extraction of caffeine from SCG was also conducted by soxhlet extraction with four organic solvents (petroleum ether, dichloromethane, n-hexane and ethanol) and water during 420 min. However, substantially lower extraction yields were obtained, namely 0.24 wt %, 0.56 wt%, 0.59 wt%, 0.74 wt% and 1.59 wt % with petroleum ether, n-hexane, dichloromethane, water and ethanol, respectively (Figure 2.8). In addition, NMR analyses of these extracts only revealed the presence of caffeine for the extraction carried out with ethanol. Therefore, higher extraction yields can be obtained with aqueous solutions of ILs at more moderate conditions.

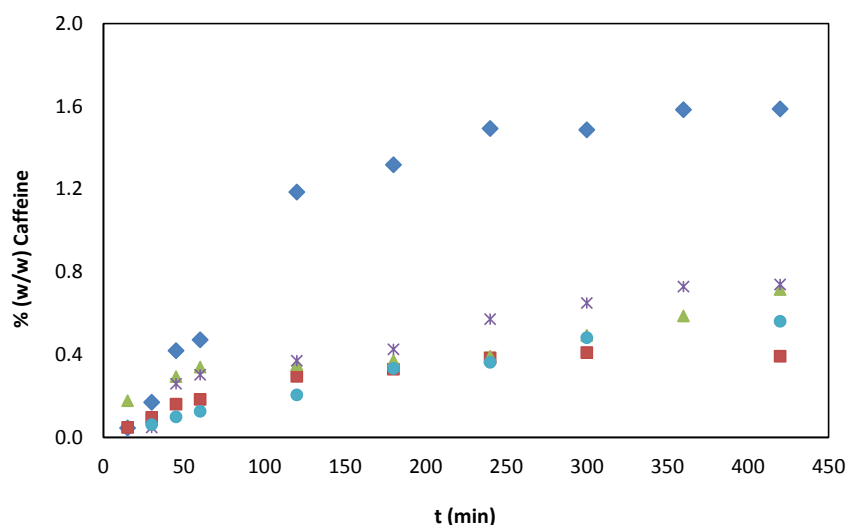


Figure 2. 8. Yield of caffeine extracted from SCG by soxhlet extraction using organic solvents and water: (♦) Ethanol, (▲) Dichloromethane, (■) Petroleum ether, (*) water, (●) n-hexane.

2.4. Conclusions

Three new PILs were synthesized, characterized, and were further used in aqueous solutions in the extraction of caffeine from SCG. From the data obtained it was confirmed that cheaper and more benign PILs can be used for the extraction of added-values compounds of biomass instead of the well-studied imidazolium-based compounds. Extraction yields of caffeine up to 3.01 wt% were achieved with an aqueous solution of 2 M of [TEA][CH₃CO₂].

By analysing the data obtained through the factorial planning of caffeine extraction using water it is possible to conclude that the determining parameters of extraction are the temperature and the solid-liquid ratio. It was found that higher amounts of caffeine extracted occur at higher temperatures and at lower solid-liquid ratios. The optimal conditions were then used in the extraction of caffeine from SCG using aqueous solutions of PILs at 2 M. By analysing the extraction data, it was proved the enhanced extractive ability of these ILs since the percentage of caffeine extracted using aqueous solutions of PILs is quite superior to that obtained using pure water at the same conditions and by Soxhlet extraction using organic solvents. The organic solvent which led to a higher percentage of caffeine was ethanol (1.59 % w/w), slightly higher than that obtained with water (1.50% w/w) in the operating optimal conditions and which is well below to that obtained using an aqueous solution of [TEA][CH₃CO₂] (3.01 % w/w).

This experimental work revealed the extractive capacity of PILs, in this particular case for the extraction of caffeine, while confirming the enhanced potential of PIL-water mixtures over conventional methods which use organic solvents and require a long time of extraction.

3. Extraction of caffeine using cholinium-based ILs aqueous solutions

3.1. Introduction

Currently, there is a great demand for natural ingredients and the discrimination between natural and synthetic caffeine has received an enormous attention.

Aiming at evaluating the toxicity of some ILs, several studies [76, 77], have been conducted. Nevertheless, this toxicity can be non-negligible and in order to overcome this major drawback, the use of non-toxic and more environmentally benign ILs from renewable materials is strongly recommended. Among these, cholinium-based salts are derived from natural resources and have emerged, in recent years, as renewable and completely bio-derived ILs [78]. Cholinium-based ILs are less expensive as a consequence of the lower cost of the cation starting material, and are more biocompatible and more biodegradable compared with more common ILs, such as imidazolium- or pyridinium-based [78]. Cholinium-based ILs are usually derived from choline chloride, also known as 2-hydroxyethyltrimethyl ammonium chloride or vitamin B4. Choline chloride can be found in food obtained from vegetable or animal sources, and supports several essential biological functions [79].

Here, the extraction of caffeine from SCG was studied, using aqueous solutions of cholinium-based ILs. Additional conditions such as the IL concentration, the solid-liquid ratio and temperature were further optimized by a response surface methodology.

3.2. Experimental section

3.2.1. Chemicals

The SCG used were described in section 2.2.1.

The ILs studied were cholinium ((2-hydroxyethyl)trimethylammonium) chloride, [Ch]Cl (99 wt% pure), cholinium acetate, [Ch][Ac] (98 wt% pure), cholinium dihydrogen phosphate, [Ch][DHPs] (99 wt% pure), cholinium dihydrogen citrate, [Ch][DHCit] (98 wt % pure), and [Ch][Bit] (98 wt % pure). [Ch][Ac] was purchased from Iolitec. The remaining ILs were acquired from Sigma-Aldrich. The following ILs were also studied and were synthesized in our laboratory according to standard protocols [80, 81], namely cholinium propanoate, [Ch][Prop], and cholinium butanoate, [Ch][But]. Before use, all the ILs were purified and dried for a minimum of 24 h at constant agitation, at moderate temperature ($\approx 60\text{ }^{\circ}\text{C}$) and under vacuum (to reduce their volatile impurities to negligible values). After this step, the purity of each IL was confirmed by ^1H and ^{13}C NMR spectra and found to be $> 98\text{ wt } \%$.

The water employed was ultra-pure water, double distilled, passed by a reverse osmosis system and further treated with a Milli-Q plus 185 water purification equipment. The chemical structures of the investigated ILs are depicted in Figure 3.1.

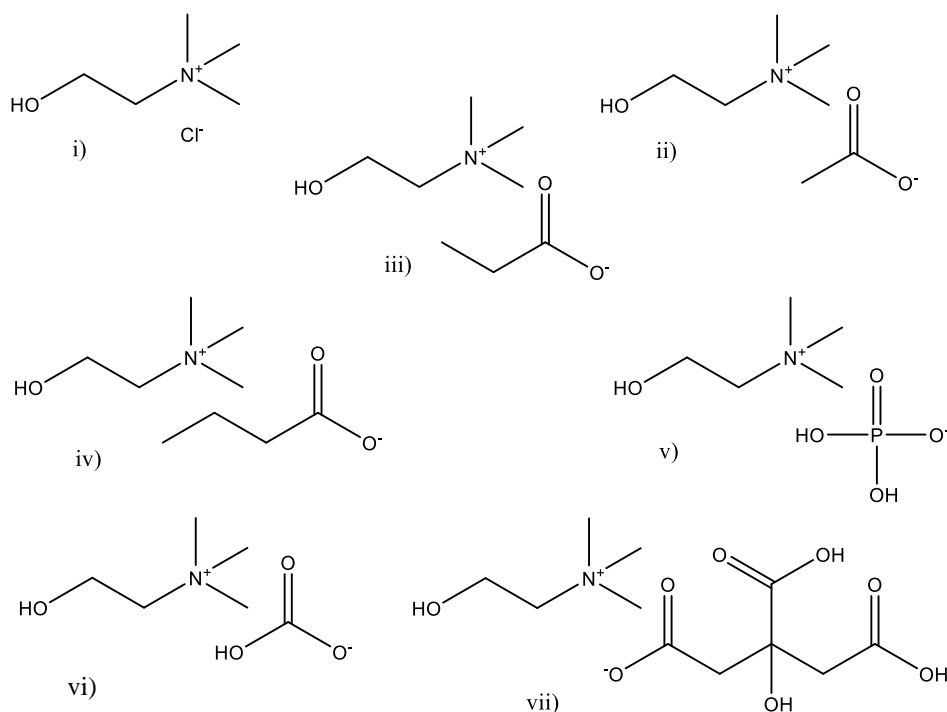


Figure 3. 1. Chemical structures of the ILs investigated: (i) [CH]Cl; (ii) [CH][CH_3CO_2]; (iii) [CH][Prop]; (iv) [CH][But], (v) [CH][DHPs], (vii) [CH][Bic] and (vii) [CH][DHCit],.

3.2.2. Experimental procedure

3.2.2.1. Caffeine Extraction

In order to extract caffeine from SCG, mixtures of specific amounts of SCG and aqueous solutions of ILs were prepared by weight and in sealed glass vials as described in section 2.2.2.3, and all equipment and conditions were the same as described before.

3.2.2.2. Surface Response Methodology

Factorial planning allows the simultaneous analysis of various factors. In this work, a 2^3 factorial planning was also used and as presented in section 2.2.2.4 to identify the most significant parameters and their interactions. This planning has been defined by the central point (zero level), the factorial points (1 and -1, level one) and the axial points (level α), described in Table 3.1. The solvent used in this planning was [Ch][Bic].

Table 3. 1. Coded levels of independents variables used in the second factorial planning.

Studied parameters	Symbol	Level				
		Axial	Factorial	Central	Factorial	Axial
		-1.68	-1	0	1	1.68
Temperature (°C)	T	24.80	35.00	50.00	65.00	24.80
Concentration (M)	C	0.16	0.50	1.00	1.50	0.16
Solid-liquid ratio	R	0.05	0.07	0.10	0.13	0.05

3.3. Results and discussion

3.3.1. Effect of the Ionic Liquid Structure

As a first screening attempt, fixed conditions were used using different ILs in aqueous media to select the best IL which leads to a higher extraction of caffeine from SCG. From Figure 3.2 it can be observed that the amount of caffeine extracted is greatly influenced by the IL anion. The ILs with anions which showed a lower capacity for extracting caffeine were composed of acetate, [Ac]⁻, and dihydrogen phosphate, [DHPh]⁻, while butanoate, [But]⁻, and bicarbonate, [Bic]⁻, lead to better extraction yields results. Anions such as dihydrogen citrate, [DHCit]⁻, and propanoate, [Pro]⁻, reveal an intermediate ability to extract caffeine. In general, extraction yields ranging from 0.88% (w/w) to 3.10% (w/w) were obtained, which can be compared with the value displayed by pure water of 1.50% (w/w) (shown in Figure 2.7). Besides its good performance, [Ch][Bic] is, among all ILs

investigated, the compound which has the lowest ecotoxicity [82], being this a remarkable feature when envisaging the possible applications of the extracted caffeine.

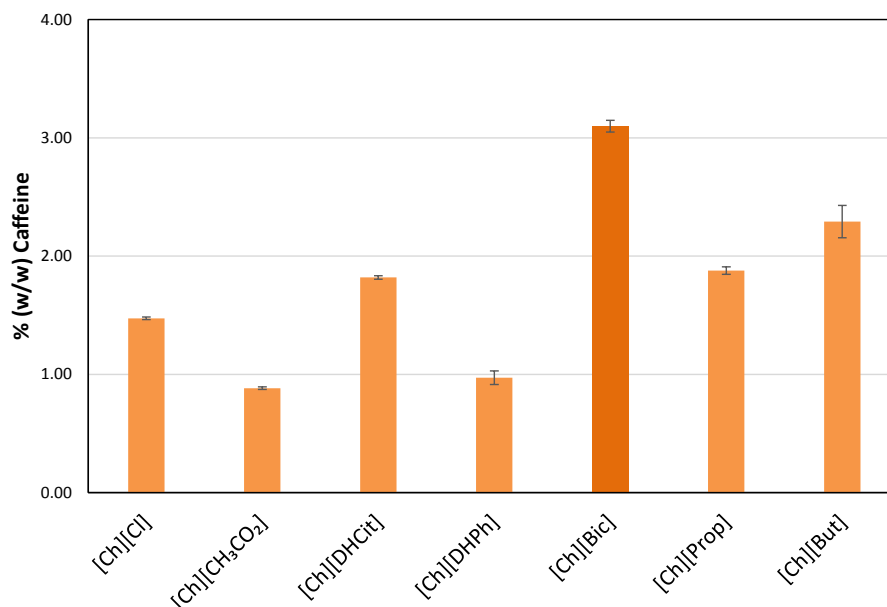


Figure 3. 2. Weight fraction percentage of caffeine extracted from spent coffee with several aqueous solutions of ILs (concentration of IL at 2 M, T = 85 °C, R =0.5:10, t = 45 min, 300 rpm).

3.3.2. Optimization of the Operational Conditions using aqueous solutions of [Ch][Bic] as main solvent

Due to the higher performance of [Ch][Bic] aqueous solutions, this IL was further used in the optimization of the operational conditions by a factorial planning. By analysing the surface charts (Figure 3.4.), it is found that the caffeine fraction is higher as higher are the operating temperatures, particularly at temperatures higher than 80°C. The same applies to the concentration of IL; when this is higher than 1 M, the higher is the extraction yield of .In relation to the solid-liquid ratio good extraction values are obtained when this ratio is low, and for values below 0.15, the caffeine extraction increases very significantly. In summary, in this optimization work it was found that the variables temperature, concentration and ratio are significant variables in the caffeine extraction process.

Figure 3.4 depicts the response surface on the extraction of caffeine using aqueous solutions of [Ch][Bic]. By these plots, it is found that temperature, although not in very high values, does not show much influence on the caffeine extraction process. Only at higher temperatures, higher than 343.15 K (70 °C), there is a substantial increase in the influence of temperature on the extraction

which may influence the statistical processing. Although the optimal conditions could lead to an extraction of caffeine of 3.10 %, this only occur at temperature higher than 353.15 K (80 °C). Therefore, the realistic optimal point, and while envisaging a low energetic consumption, should be that with a low energy consumption as well as with a lower waste of resources. Thus, the optimal point (temperature of 323.15 K (50 °C), ratio solid-liquid 0.05, IL concentration of 1.5 and time of 30 minutes) leads to an extraction of 1.85% (w/w) of caffeine where the mathematical model predicted a value of 1.98% (w/w).

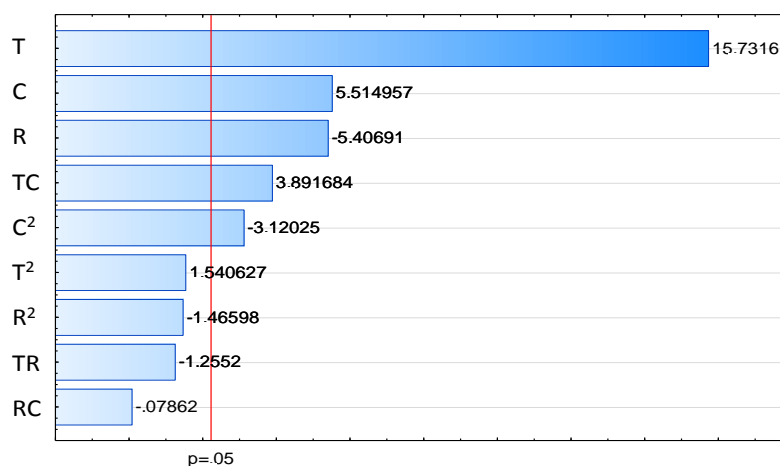


Figure 3.3. Representation of optimization variables: T (temperature), C (concentration of IL aqueous solution), R (ratio S/L) Pareto chart. $R^2 = 0.9713$; $\text{adj-}R^2 = 0.946$; p-value = 0.000002.

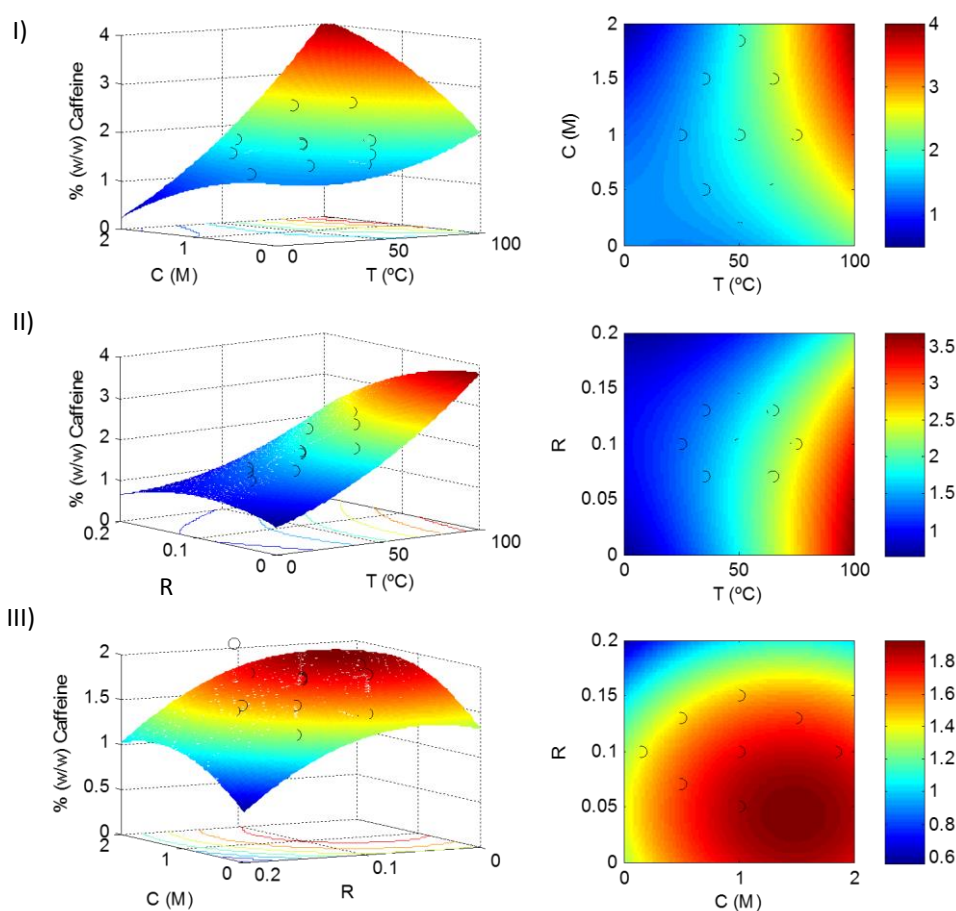


Figure 3. 4. Response surface on the extraction of caffeine using aqueous solutions of [Ch][Bic] ($t = 30$ min: (I) T and C; (II) C and T; (III) T and R).

3.4. Conclusions

This work showed the extraction capacity of caffeine from spend coffee grounds using aqueous solutions of cholinium-based ILs. By the analysis of the data obtained through factorial planning it can be concluded that temperature is the most significant parameter, followed by the solid-liquid ratio and concentration of IL. As in the previous planning factor, using pure water, higher temperatures and lower solid-liquid ratios favor the extraction of caffeine. Moreover, higher concentrations of ILs lead to higher extraction yields.

Aqueous solutions of ILs also display a higher extraction capacity for caffeine than the organic solvents commonly used and studied in the previous chapter. The cholinium-based IL studied with the best performance was [Ch][Bic] because it can extract a higher percentage of caffeine, 3.10 % (w/w) at the optimal conditions and it is the one with the lowest ecotoxicity amongst the studied ILs.

Although values of extracted caffeine up to 3.10 % (w/w) can be obtained, the optimum point would be that in which maximum good amount of caffeine is obtained combined with a proper combination of the operational conditions, namely temperature and concentration of IL. From a critical perspective, the optimal conditions correspond to $T=50^{\circ}\text{C}$, $R=0.05$ and $C=1.5\text{ M}$, where the percentage of caffeine extracted is 1.85% (w/w).

The great advantage of using an IL with low ecotoxicity and high capacity for the extraction of caffeine from biomass relays on possible applications of the extract in food supplements or even in pharmacology.

**4. Extraction using mixtures
of salt + IL Extraction of
caffeine using aqueous
solutions composed of ILs and
salts**

4.1. Introduction

In addition to caffeine, coffee also contains other compounds, such as polysaccharides, lignans, polyphenolics, lipids, melanoidins and chlorogenic acid [8]. Caffeine is known to interact directly with some of these constituents, such as the chlorogenic acid, to which it weakly binds [83]. While caffeine has significant dipole and quadrupole moments, its flat and weakly-hydrating non-polar faces make it only sparingly soluble in water at room temperature [84], although it becomes more soluble at higher temperatures. Due to these valuable features, more efficient solvents for the extraction of caffeine from spent coffee should be investigated.

In recent years, several authors have debated the concept of the ionicity of ILs [85, 86], i.e., the relationship between ion concentration and ion mobility in a fluid medium. Ionicity is controlled and serves as an effective way to probe the balance between different interactive forces – electrostatic, dispersive, hydrogen-bonding, and $\pi\cdots\pi$ – present in ILs. The ionicity concept can also be used to analyse the IL structure since the association of ions as ion pairs or other charged/non-charged aggregates will also impact the effectiveness of ion mobility via altered conductivity and diffusion coefficients. Other properties, such as vapour pressure and hydrogen acceptor or hydrogen donor character, can also be correlated to the ionicity concept [87].

Hydrotropes are compounds used to increase the concentration levels of hydrophobic solutes in aqueous solutions. Their ability to dramatically increase the solubility of sparingly soluble organic compounds in water was already demonstrated [88, 89]. In addition to the increase on the solubility, hydrotropes may also play a role in the stabilization of aqueous solutions, on the tailoring of their viscosity, and by modifying the liquid-liquid phase separation temperatures [90]. They are interesting compounds from a green chemistry perspective as they can replace hazardous co-solvents and display, in general, a low toxicity and have a low bioaccumulation potential - their octanol-water partition coefficients, due to their hydrophilic nature, are usually lower than 1.0 [91]. Moreover, and since the solubility of a solute in aqueous media depends on the concentration of the hydrotrope, their recovery can be easily achieved using water as the anti-solvent (the greenest of all solvents). In this context, the purity of the final product can be greatly improved by further washing with water [92]. In this context, in this work, it is investigated the potential of aqueous solutions composed of ILs and hydrotrope salts, while taking advantage of each type of compounds features, to extract caffeine from spent coffee.

4.2. Experimental section

4.2.1.1. Chemicals

The SCG used were described in section 2.2.1.

The salts studied were Na[Tos] (sodium p-toluenesulfonate, purity > 90%) was supplied from Alfa Aesar, NaCl (sodium chloride), Na[CH₃CO₂] (sodium acetate) was acquired from Chem Lab, Na[SCN] (sodium thiocyanate, purity ≈ 98.0%) was obtained from Fluka, and Na[N(CN)₂] (sodium dicyanamide). The ILs studied were [C₄mim][Tos] (1-butyl-3-methylimidazolium tosylate, purity ≈ 98.0%), [C₄mim]Cl (1-butyl-3-methylimidazolium chloride purity ≈ 99.0%), [C₄mim][CH₃CO₂] (1-butyl-3-methylimidazolium acetate), and [C₄mim][N(CN)₂] (1-butyl-3-methylimidazolium dicyanamide, purity ≈ 98.0%). The ILs were purchased from Iolitec.

4.2.1.2. Experimental procedure

In order to extract caffeine from SCG, mixtures of specific amounts of SCG and aqueous solutions were prepared by weight in specific sealed glass vials as described in section 2.2.2.3. The equipment and conditions were the same as described in that section. The only difference in this particular extraction is that aqueous solutions contain now a mixture of salts and ILs.

Initial extractions were made from SCG using separately aqueous solutions of salts and commercial ILs. After analysing what were the compounds with higher caffeine extraction capacity, mixtures of salts + ILs were the subject of study.

4.3. Results and discussion

The analysis of the results reported in Figure 4.1 show a comparison on extractions made using ILs with extractions using salts aqueous solutions, and it was found that ILs containing the same anion as the sodium salts are better extraction solvents for caffeine, with an exception in the case of compounds containing the tosylate anion for which a reverse trend occurs.

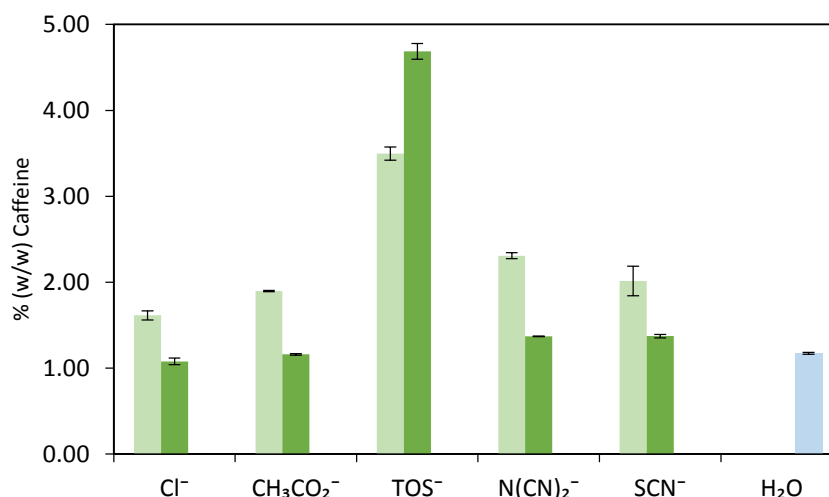


Figure 4. 1. Extraction of caffeine using ILs and sodium salts with the same anion at concentration of 1 M.

(■) C₄mim⁺ (■) Na⁺ (■) H₂O. (T = 75 °C, R = 1:10, t = 30 min, 300 rpm).

The compounds containing the tosylate anion have very high extraction capacity, 3.50 % (w/w) in the case of [C₄mim][Tos] and 4.67 % (w/w) in the case of Na[Tos]. The compounds containing the anion chloride did not show a great capacity for the extraction of caffeine, with 1.61 % (w/w) for aqueous solutions of [C₄mim]Cl and 1.08 % (w/w) for NaCl (similar to values obtaining using pure water in the same operating conditions).

As expected, increasing the concentration of ILs and salts, there is an increase in their caffeine extraction capacity as observed in Figure 4.2. Furthermore, comparing mixtures of NaCl with [C₄mim][Tos], and Na[Tos] with [C₄mim]Cl (Figure 4.2), it is observed that at small concentrations (0.25 M) these mixtures have substantially the same ability to extract caffeine. From a chemical point of view there are the same chemical species in aqueous media. Nevertheless, aqueous solutions of Na[Tos] lead to a higher extraction yield of caffeine.

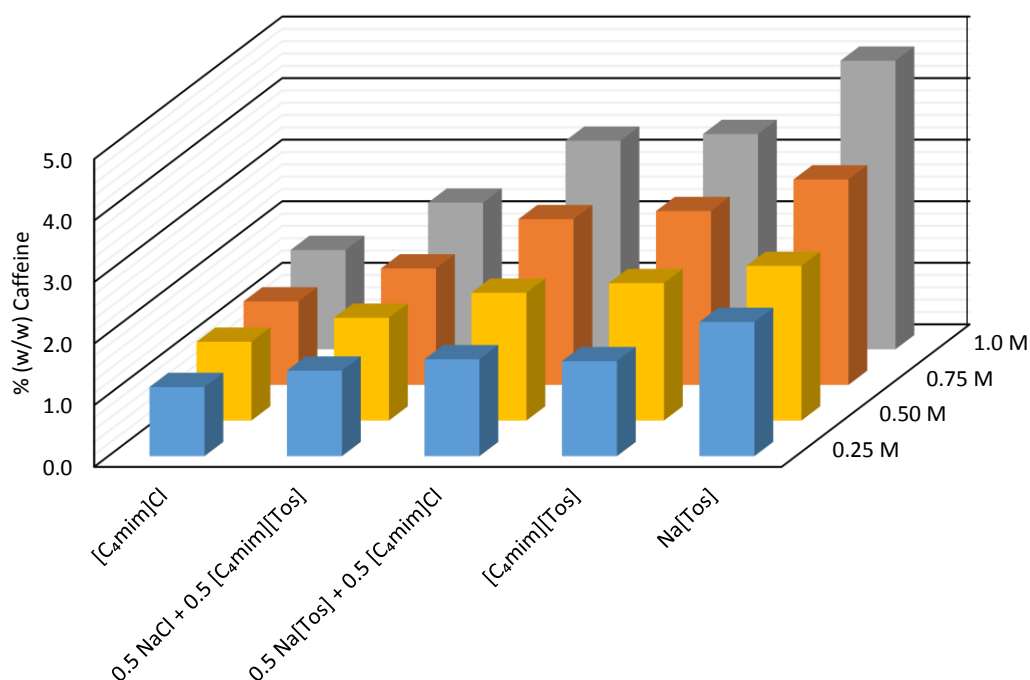


Figure 4. 2. Yield of caffeine extracted using ILs and salts, and their mixtures, at different concentrations ($T = 75\text{ }^{\circ}\text{C}$, $R = 1:10$, $t = 30\text{ min}$, 300 rpm).

4.4. Conclusions

It can be concluded that, in most cases, ILs have a higher caffeine extraction capacity when compared to the salts, except in the case for which the anion tosylate is present. In fact, with an aqueous solution of Na[Tos] at 1.0 M, 4.69 % of caffeine can be extracted from spent coffee. [C₄mim][Tos] and the equimolar mixture of Na[Tos] with [C₄mim]Cl lead to similar values of caffeine extraction, although lower caffeine yields are observed with the mixture formed by NaCl and [C₄mim][Tos]

In all extractions it was observed an increase in the caffeine extracted with the increase of salt or IL amount in aqueous media.

5. Final remarks

5.1 Conclusions

The present work showed that it is possible to extract high amounts of caffeine from spent coffee grounds using aqueous solutions of ILs and/or salt and therefore to add value to this residue.

The PILs used for extract caffeine from spent coffee grounds have demonstrated a high extraction capacity, in particular the [TEA][CH₃CO₂] when the extraction occurs at a high temperature and low solid-liquid ratios.

The most advantageous cholinium-based ILs identified was the [Ch][Bic], because in addition to being able to extract a high percentage of caffeine 3.10 % (w/w) it is the IL with the lowest ecotoxicity.

Regarding the study of ILs and hydrotrope salts, compounds containing the tosylate anion display a very high extraction capacity, 3.50% (w/w) in the case of [C₄mim][Tos] and 4.69% (w/w) in the case of Na[Tos]. This study confirms that the presence of hydrotropes in addition to increase the solubility of poorly soluble organic compounds in water also promote their extraction from bioresources.

In summary, it can be concluded that most of the aqueous solutions of ILs and salts have a higher extraction capacity than the organic solvents commonly used, and by soxhlet extractions.

5.2 Future work

As further work it should be carried out additional factorial plannings to optimize the extraction of caffeine using PILs and hydrotrope salts. Moreover, the development of a novel strategy to remove caffeine from the aqueous solution should be attempted. Later, the components used in the extraction of caffeine should undergo toxicological analysis in order to determine the possible use of the extracts in pharmaceutical and food industries.

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Appendix A

2^3 factorial planning

Table A. 1. 2³ factorial planning.

	X ₁	X ₂	X ₃
1	-1	-1	-1
2	1	-1	-1
3	-1	1	-1
4	1	1	-1
5	-1	-1	1
6	1	-1	1
7	-1	1	1
8	1	1	1
9	-1.68	0	0
10	1.68	0	0
11	0	-1.68	0
12	0	1.68	0
13	0	0	-1.68
14	0	0	1.68
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0

Table A. 2. Conditions used for the extraction of caffeine using water (2^3 factorial planning).

	T (K)	t (min)	R
1	328.15	15	1:14
2	328.15	15	1:14
3	328.15	45	1:14
4	328.15	45	1:14
5	328.15	15	1:8
6	328.15	15	1:8
7	328.15	45	1:8
8	328.15	45	1:8
9	328.15	30	1:10
10	328.15	30	1:10
11	328.15	5	1:10
12	328.15	55	1:10
13	328.15	30	0.5:10
14	328.15	30	1.5:10
15	328.15	30	1:10
16	328.15	30	1:10
17	328.15	30	1:10
18	328.15	30	1:10
19	328.15	30	1:10
20	328.15	30	1:10

Appendix B

Additional experimental data

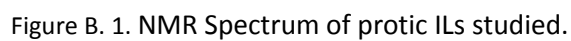


Table B. 1. Different properties of protic ILs studied.

Temperature / K	[EA][CH ₃ CO ₂]			[DEA][CH ₃ CO ₂]			[TEA][CH ₃ CO ₂]		
	n_D	η / mPa.s	ρ / g.cm ⁻³	n_D	η / mPa.s	ρ / g.cm ⁻³	n_D	η / mPa.s	ρ / g.cm ⁻³
288.15	1.482593			1.481466			1.488035		
298.15	1.480000	2329.20	1.1518	1.478951	3456.80	1.1773	1.485446		
303.15		1532.10	1.1490		2213.70	1.1741			
308.15	1.477414	1037.50	1.1462	1.476391	1460.40	1.1709	1.482774		
313.15		720.63	1.1434		989.07	1.1676		630.24	1.1703
318.15	1.474757	512.12	1.1405	1.473718	684.60	1.1643	1.480029	436.51	1.1666
323.15		371.71	1.1377		483.64	1.1610		310.08	1.1630
328.15	1.472089	274.98	1.1349	1.471056	348.65	1.1577	1.477272	224.73	1.1594
333.15		207.07	1.1321		256.12	1.1544		166.06	1.1558
338.15	1.469410	158.46	1.1294	1.468307	191.38	1.1511	1.474466	125.04	1.1523
343.15		123.06	1.1267		145.77	1.1479		95.63	1.1487
348.15	1.466673	96.94	1.1239	1.465497	112.36	1.1446	1.471649	74.41	1.1451
353.15		77.26	1.1211		87.82	1.1413		58.61	1.1414
358.15	1.463956	62.31	1.1183	1.462714	69.52	1.1380	1.468836	46.75	1.1376
363.15		50.77	1.1155		55.64	1.1346		37.71	1.1338
368.15		41.74	1.1128		45.01	1.1313		30.73	1.1299

